CLINICAL TRIAL PLAN

Treatment of Tinnitus; PR172190 Award

April 17 2019

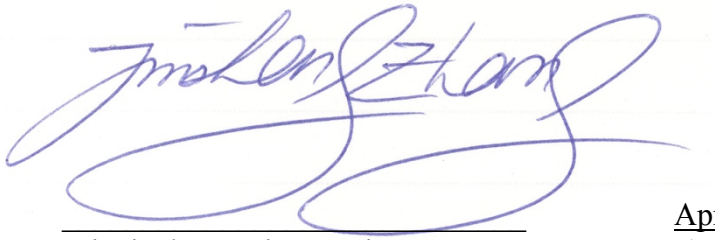
Study Sponsor: CDMRP, Department of Defense

Clinical Trial of Etanercept (TNF-α Blocker) for Treatment of Blast-Induced Tinnitus

PR172190 Award

PRINCIPAL INVESTIGATOR'S COMPLIANCE STATEMENT

By signing this protocol, I confirm that I have read and agree to conduct the trial as outlined in the protocol and in compliance with the International Committee of Harmonization and Good Clinical Practice and all other applicable regulatory requirements. Confidentiality of all information received or developed in connection with this protocol will be maintained by myself as well as all other personnel involved in the trial.



_____ April 17, 2019

Principal Investigator Signature (Date)

JINSHENG ZHANG

_____

Principal Investigator Name (Printed)

INVESTIGATOR AGREEMENT

Clinical Trial of Etanercept (TNF-α Blocker) for Treatment of Blast-Induced Tinnitus

The signature below represents my approval of:

Protocol Version Date April 17, 2019

Signature: _____ Date: _____

Site Principal Investigator: _____

Signature: _____ Date: _____

Site Investigator Statement

By signing this protocol, I confirm that I have read and agree to conduct the trial as outlined in the protocol and in compliance with the International Committee of Harmonization and Good Clinical Practice and all other applicable regulatory requirements. Confidentiality of all information received or developed in connection with this protocol will be maintained by myself as well as all other personnel involved in the trial.

Title: Clinical Trial of Etanercept (TNF-α Blocker) for Treatment of Blast-Induced Tinnitus

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PROTOCOL SYNOPSIS

Trial Phase:	II
Protocol Title:	Clinical Trial of Etanercept (TNF-α Blocker) for Treatment of Blast-Induced Tinnitus
Objective:	The objective of this clinical trial is to determine if Etanercept significantly reduces the severity of tinnitus associated with blast- or noise-exposure and yields persistent benefit, elucidating the mechanisms of action, and to identify factors that influence the therapeutic effects of Etanercept.
Subject Population:	310 subjects who have blast- or noise trauma-induced tinnitus.
Design:	This is a multi-center, prospective, randomized, double blinded, saline placebo-controlled study of Etanercept administered subcutaneously. For mechanistic studies, a subset of 40 subjects will undergo imaging including resting-state fMRI, DTI, and SWI at pre-treatment, 12 weeks during treatment, and 24 weeks after the last treatment. Blood serum tests will be administered on another subset of 60 subjects (30 each at a VA and civilian site) to monitor TNF-α concentration at pre-treatment, 1, and 12 weeks during treatment.
Number of Sites:	7 sites with enrollment; 2 sites without enrollment
Dosing Schedule:	After completing all clinical evaluations, 50 mg of Etanercept or placebo will be injected subcutaneously weekly for 12 consecutive weeks, based on the manufacturer's recommendation and clinical guideline. The proposed trial will utilize a placebo control design that includes the following elements: (1) a saline placebo in an identical syringe; (2) blinding of all subjects and research team members who administer outcome assessments. Baseline assessments will be conducted after informed consent is signed prior to the start of treatment, and outcome measures will be made at 1, 4, 8, and 12 weeks during the 12 weekly treatments. Follow-up evaluations will be conducted at 4, 8, 12, and 24 weeks after the last treatment session, this will be the same as study schedule week 16, 20, 24 and 36 respectively.
Inclusion Criteria:	• Tinnitus associated with blast- or noise-exposure of at least a moderate severity as defined by a score of ≥ 25 points or higher on the Tinnitus Functional Index (TFI) questionnaire[1], and a self-rated visual numeric score (VNS) of at least 5 out of 10 for tinnitus loudness. • Able to provide written informed consent. • Age/Gender: Minimum 18 years of age at the time of enrollment. • Other concurrent treatments: A four-week washout from any other tinnitus treatment or management program is required prior to entering this study.

	• Psychological status: Stable enough to complete this study per the opinion of the research team. • Hearing function: All degrees of hearing function can be included, recognizing that individuals with profound, bilateral hearing loss will not be able to perform tinnitus evaluations and hearing tests but will be able to rate subjective tinnitus loudness, annoyance and impact on life. This is an important sub-population because of the challenges in treating them with acoustic therapy and the need for a medical intervention. • Additional tinnitus characteristics: o *Tinnitus history:* Associated with history of blast or noise exposure. o *Stability:* Constant (not pulsatile, intermittent, varying to a high degree in loudness or changing in location of perception). o *Location of tinnitus perception:* Unrestricted. Tinnitus may be unilateral, bilateral, or perceived in the head.
Exclusion Criteria:	• History or evidence of significant brain malformation or neoplasm, cerebral vascular events (such as strokes), neurodegenerative disorders affecting the brain (such as Parkinson's disease, ALS, Huntington's disease or Multiple sclerosis), or prior brain surgery. • History of seizures or epileptic activity. • Subjects with cardiac pace makers, other electronic implants (including cochlear implants), or intracranial or intraocular metallic particles. • Subjects who currently have an active infection, including tuberculosis and chicken pox. • Diagnosis of active neurologic disease, auto-immune disease, a weak immune system, diabetes, HIV, hepatitis B, or current or past heart failure. • Ongoing treatment with contraindicated medications: abatacept, cyclophosphamide or sulfasalazine. • Subjects who cannot communicate reliably with research team members or who are not likely to cope with the requirements of the trial. • Subjects who have participated in a clinical drug trial within the last 30 days before the start of this one. • Current substance abuse (defined as a score of 2 or greater on the CAGE Substance Abuse Screening Tool) • Pregnancy or planned pregnancy during the study. • Women who are lactating or are of child-bearing-age without use of contraception.

	• MMSE score less than 24
Safety Evaluations:	Subjects will be followed for identification and frequency of drug or procedure-related adverse events. Any clinically significant changes in the medical assessment of the subject will be documented and reported.
Data Analysis:	All adverse events will be reported by tabular description and will specify whether they occurred during or after dosing. Severity and physician attribution as to likelihood of association to study drug will also be reported. Any effect of the drug on tinnitus will be summarized using descriptive statistics.

SCHEDULE OF EVENTS

	Pre-Treatment	Treatment Phase				Follow-up Phase			
Study Procedures	**Baseline -14 days**	**Week 1**	**Week 4**	**Week 8**	**Week 12**	**Week 16**	**Week 20**	**Week 24**	**Week 36**
Informed Consent and HIPAA authorization	X								
Physical Exam	X								
Vital signs (all visits inclusive of weekly injections)	X	X	X	X	X	X	X	X	X
Pregnancy Test (if appropriate)	X								
Tuberculosis Test	X								X
Laboratory Testing - CBC & Electrolyte Panel	X		X		X				
Medical History Questionnaire	X								
Tinnitus History Questionaire	X								X
CAGE Screening Tool	X								
Blast Questionnaire	X								
Mini Mental State Examination (MMSE)	X								
Speech Spatial & Qualities of Hearing (SSQ-12)	X				X			X	X
Tinnitus Functional Index (TFI)	X		X	X	X	X	X	X	X
Tinnitus Primary Function (TPF) Questionnaire	X				X			X	X
Hospital Anxiety and Depression Scale (HADS)	X				X			X	X
Short-Form Health Survey 12 items (SF-12)	X				X			X	X
Audiologic Testing (see below)									
Otoscopy	X	X	X	X	X	X	X	X	X
Pure tone air conduction (AC) testing	X	X	X	X	X	X	X	X	X
Pure tone bone conduction (BC) testing	X	(X)[a]	(X)[a]	(X)[a]	(X)[a]	(X)[a]	(X)[a]	(X)[a]	(X)[a]
Speech Reception Threshold (SRT)	X	X	X	X	X	X	X	X	X
Tympanometry	X	(X)[b]	(X)[b]	(X)[b]	(X)[b]	(X)[b]	(X)[b]	(X)[b]	(X)[b]
Word Recognition Testing	X				X				X
Tinnitus Loudness Matching	X	X	X	X	X	X	X	X	X
Minimum Masking Levels (MML)	X	X	X	X	X	X	X	X	X
Visual Numeric Scale (VNS)	X	X	X	X	X	X	X	X	X
Blood serum testing*	X	X			X				
MRI testing**	X				X			X	
AE/SAE Assessment	X	X completed at weekly visits				X	X	X	X
Concomitant Medication	X	X completed at weekly visits				X	X	X	X
Study Drug Administration		weekly injections for 12 consecutive weeks							

[a] BC performed at baseline and repeated at future visits only if >10 dB change in AC thresholds observed at one or more frequencies

[b] Tympanometry performed at baseline, then at future visits only if there is an air bone gap of 15 dB or greater at two adjacent frequencies, or 20 dB or greater at any one frequency

*subset of participants (n=60) will have blood drawn

**subset of participants (n=40) will undergo imaging To be conducted at Wayne State University MRI research facility

LIST OF ABBREVIATIONS

AD	axial diffusivity
ADE	adverse drug effects
ADR	adverse drug reactions
AE	adverse event
ASHA	American Speech-Language-Hearing Association
BDI	Beck depression inventory
BOLD	blood oxygen level dependent
CNS	central nervous system
CRF	case report form
CSF	cerebrospinal fluid
DAN	dorsal attention network
DMN	default mode network
DTI	diffusion tensor imaging
ELISA	enzyme-linked immunosorbent assay
ENT	Ear, Nose & Throat
FA	fractional anisotropy
fMRI	functional magnetic resonance imaging
Hz	Hertz
kHz	kilohertz
KO	knockout
MD	mean diffusivity
MMLs	minimum masking levels
MMSE	Mini-Mental State Examination
pCASL	Pseudocontinuous arterial spin labeling
RD	radial diffusivity
ReHo	regional homogeneity
rCBF	regional cerebral blood flow
SAE	serious adverse event
SPL	sound pressure level
SWI	susceptibility weighted imaging
TBI	traumatic brain injury
TFI	tinnitus function index
TNF	tumor necrosis factor
TPF	tinnitus primary function
VNS	visual numeric scale

TABLE OF CONTENTS

STUDY PROTOCOL

1.0 OBJECTIVES AND HYPOTHESIS

1.1 Trial Objectives

The primary objectives are to test if: 1) Etanercept significantly reduces tinnitus distress as measured by Tinnitus Functional Index (TFI) and Tinnitus Primary Function (TPF) scores; 2) Etanercept improves hearing; and 3) Etanercept reduces tinnitus distress by restoring abnormal functional connectivity between auditory and limbic brain structures to physiological levels, as revealed by resting-state fMRI. In addition, we will test if: 1) Etanercept treatment leads to sustained therapeutic effects over time; 2) Etanercept-induced tinnitus relief is accompanied by restored abnormal functional connectivity between brain centers, extending past cessation of treatment; 3) Etanercept will further improve microvasculature-related neural plasticity after treatment.
Our secondary objectives are to test if: 1) Etanercept reduces tinnitus loudness measured by visual numeric scale (VNS) rating; 2) Etanercept decreases TNF-α concentration in blood serum; 3) Etanercept reduces tinnitus distress and/or loudness by improving maladaptive, microvasculature-related neural plasticity.

1.2 Central Hypothesis

Blocking TNF-α reduces the severity of tinnitus for prolonged periods of time.

2.0 BACKGROUND AND INFORMATION

2.1 Tinnitus

Tinnitus is a condition whereby individuals perceive relentless phantom sounds in the absence of physical acoustic stimulation and a cure remains elusive. It is a significant problem for military personnel and Veterans, as blast or loud noise exposure can induce the condition. In the Iraq and Afghanistan wars, blast-related trauma is the "signature injury" and chronic tinnitus and hearing loss are the most common auditory-related co-morbidities[2]. Furthermore, since 2007, tinnitus has remained the top service-connected disability for Veterans receiving compensation. For affected individuals, tinnitus can impact daily life by inducing anxiety, annoyance, irritability, disturbed sleep patterns and depression[3-9]. These facts together illustrate the urgency for developing an effective therapy to abate and eventually cure this condition. Among a number of candidate treatments, pharmacological targeting of the TNF-α cytokine has extremely high potential.

2.2 Specific aims of the clinical trial

Noise-induced hearing loss and tinnitus are prevalent problems, and can have debilitating consequences. The economic impact of tinnitus is high, thus, developing effective therapy, especially on a pharmacological level, is urgently needed. This phase II clinical trial will examine the therapeutic effects of Etanercept (Enbrel®), a tumor necrosis factor α (TNF-α) blocker, on tinnitus associated with noise exposure (FDA-IND exempted #137226). In

humans, it has been shown that TNF-α contributes to autoimmune cochleovestibular disorders including hearing loss and tinnitus. A number of studies suggest that blocking TNF-α not only down-regulates excessive inflammatory responses but also modulates maladaptive neural plasticity that manifests in response to chronic stress from hearing loss or tinnitus associated with noise trauma. Recently, Etanercept was used to treat microcirculation-related hearing loss in humans after noise trauma. The available evidence prompts the feasibility of conducting this clinical trial and testing the central hypothesis: that blocking TNF-α can reduce the severity of tinnitus for prolonged periods of time, via acting on the immune system and mediating neural plasticity. We will test our central hypothesis and accomplish the objective of this study by pursuing the following specific aims:

Specific Aim 1: Test for the therapeutic effect of Etanercept on noise-induced tinnitus in a double-blinded, randomized, placebo-controlled clinical trial. We will test three primary hypotheses that Etanercept: 1) significantly relieves tinnitus distress as measured by Tinnitus Functional Index (TFI) and Tinnitus Primary Function (TPF) scores; 2) improves hearing as seen by improved hearing thresholds and word recognition ability; and 3) reduces tinnitus severity by restoring tinnitus-related abnormal functional connectivity between brain structures, as revealed by resting-state fMRI. Our secondary hypotheses are that Etanercept: 1) reduces tinnitus loudness as measured by the self-rated visual numeric scale (VNS) and 1 kHz loudness-matches; 2) decreases TNF-α concentration in blood serum, and 3) reduces tinnitus severity by improving microvasculature-related neural plasticity. To test these hypotheses, subjects with tinnitus associated with blast and/or noise exposure will receive 12 consecutive weekly treatments of Etanercept. Outcome measures include TFI and TPF questionnaires, and audiometric and tinnitus tests at baseline, and weeks 1, 4, 8 and 12, with the primary end point at 12 weeks. For a subset of subjects, we will monitor TNF-α concentration changes, and use resting-state fMRI protocols for assessing functional connectivity changes, diffusion tensor imaging (DTI) for white matter integrity, and susceptibility weighted imaging (SWI) for microvasculature-related neural plasticity. The results will help to determine the therapeutic effects of Etanercept on noise trauma-induced tinnitus and hearing impairment, and elucidate the underlying mechanisms of the intervention.

Specific Aim 2: Investigate the time course of the therapeutic effect of Etanercept on tinnitus associated with blast and/or noise exposure. In this aim, there are three primary hypotheses: 1) Etanercept treatment will maintain the therapeutic effects over time; 2) Relief from tinnitus distress will be accompanied by restored functional connectivity between brain centers; and 3) improved microvasculature-related neural plasticity will occur post-treatment. To test these hypotheses, we will administer audiological and tinnitus tests, and TFI and TPF questionnaires to examine the therapeutic effects of Etanercept on tinnitus severity during the follow-up sessions. Specifically, following the 12 weeks of Etanercept treatment, each subject will undergo post-treatment outcome assessments at 4, 8, 12 and 24 weeks after the final administration of Etanercept, this will be the same as study schedule week 16, 20, 24 and 36 respectively. At each post-treatment appointment, subject will undergo the same tinnitus scoring and audiometric tests. For a subset of subjects, resting state fMRI, DTI, and SWI will be conducted to determine whether Etanercept-induced tinnitus relief is accompanied by restored white matter integrity,

microvasculature-related plasticity, and functional connectivity changes between brain centers. The information gleaned help determine the efficacy of Etanercept treatment for tinnitus.

Exploratory Aim 3: Identify factors that influence the therapeutic effects of Etanercept on tinnitus associated with blast and/or noise exposure. We will conduct exploratory investigations to identify factors that influence the therapeutic effects of Etanercept on tinnitus associated with blast and/or noise exposure. These factors, which can lead to subgrouping of subjects, include the following variables: 1) Age; 2) Hearing sensitivity; 3) History of noise exposure; 4) Time since noise exposure (and number of noise exposures); 5) Time since tinnitus started (tinnitus duration); and; 6) History of traumatic brain injury (TBI). These variables will be used to characterize the therapeutic effects of Etanercept. Although the current proposal focuses on tinnitus as a result of noise trauma, various additional etiologies may present and be considered across different tinnitus subjects. In this aim, the same outcome measures as illustrated above will be evaluated. The results will allow us to identify sub-groups of tinnitus sufferers so that optimal treatment strategies can be developed.

2.3 Impact of developing Etanercept to effectively treat tinnitus

Blast- and noise-induced injuries, including hearing loss, tinnitus, limbic dysfunctions, and traumatic brain injury (TBI) are commonly identified medical problems among military personnel and Veterans. If left untreated, tinnitus can have debilitating effects on emotional and psychological well-being, and disrupt daily life. Unfortunately, tinnitus-related disability compensation to Veterans costs the Department of Veterans Affairs hundreds of millions of dollars per year, creating a significant economic burden on society.

Currently, there is no clinically-proven cure for tinnitus. Tinnitus management and treatment options do exist, including counseling, relaxation therapy, habituation therapies, tinnitus maskers, biofeedback, hypnosis, and electrical stimulation. However, most of these treatment/management methods are ineffective, inconsistent, and inconvenient. Furthermore, there are no FDA- or even EMA-approved pharmacological treatments for tinnitus. Developing an effective drug treatment for tinnitus associated with blast- and/or noise exposure will significantly improve the quality of life for military personnel and Veterans, and relieve the economic burden on the US government and society. It would also have a large market in civilian patients.

The proposed research will test Etanercept for the treatment of tinnitus associated with blast and/or noise-exposure. It is based on emerging literature and recent results of DoD-funded research indicating a key role of TNF-alpha, a proinflammatory cytokine, in tinnitus pathology. The proposed project is a multi-site, double-blind, placebo-controlled clinical trial. It will provide definitive conclusions for using Etanercept, a prescription drug, to treat tinnitus. If proven effective, Etanercept can quickly be developed for clinical use for tinnitus and provide much needed relief for affected military personnel and Veterans, as well as for civilian tinnitus sufferers.

The target population of the proposed intervention is military personnel, Veterans, and civilians who suffer from acute or chronic tinnitus associated with blast exposure and/or noise trauma. The volunteer population(s) that will participate in the intervention will be recruited through military and Veteran's hospitals as well as other established otolaryngology and/or audiology clinics.

***The short-term impact**:*

The proposed clinical trial will be the first time to test a TNF-alpha blocker as a treatment option for tinnitus. If successful, it will provide strong support for treating tinnitus by blocking TNF-alpha, and will point to a new direction (TNF-alpha signaling) in tinnitus research, and will accelerate drug discovery. Pharmacological treatment of tinnitus has long focused on modulators of channels and receptors that directly modulate neuronal excitability. However, this approach has not been successful. As our understanding has evolved, we now know that neuronal excitability is not controlled by a single channel or receptor. Instead it is controlled by many channels and receptors through a coordinated process known as homeostatic plasticity. Homeostatic plasticity is intricately related to neural inflammation, and TNF-alpha is a central modulator of homeostatic plasticity and neural inflammation. It cohesively modulates many channels and receptors to in turn modulate neuronal excitability. It therefore represents a better target for tinnitus treatment. The current clinical studies will provide the first evidence for the treatment of HUMAN tinnitus with TNF-alpha blockers.

The proposed clinical trial will examine potential contributing factors to the efficacy of Etanercept on tinnitus. It has long been known that tinnitus is not a homogeneous symptom, and there are subgroups of tinnitus that may be treated more efficiently by different drugs. Such a concept has not been fully explored in clinical studies. We thus propose to investigate how physical/health/clinical factors influence the efficacy of Etanercept on tinnitus. The identification of specific patient populations that are differentially responsive to drug treatment would have strong impact on research and clinical treatment of tinnitus.

The proposed clinical trial will examine, in a subset of the patients, whether systemic TNF-alpha level is a biomarker, or a relevant clinical measurement for tinnitus treatment. It has been reported that blood TNF-alpha level is correlated with tinnitus severity. The proposed study provides an opportunity to examine a causal relationship between the two by perturbing the systemic TNF-alpha level and examining its effects on tinnitus.

The proposed clinical trial will use brain-imaging techniques to examine how Etanercept treatment alters putative brain pathologies related to tinnitus. The impact here will be two-fold. First, alleviation of brain pathologies by Etanercept would provide corroborating evidence that Etanercept impacts tinnitus. Second, correlated modulation of tinnitus symptoms and the putative brain pathologies would provide evidence that the brain pathologies are in fact causally related to tinnitus. This would provide insights on the mechanisms of tinnitus and shed new light on tinnitus treatment.

***The long-term impact**:*

If Etanercept is proven effective in the proposed clinical trial, it could be developed into a first-in-class drug to treat tinnitus. Etanercept is a prescription drug for rheumatoid arthritis and autoimmune skin diseases. It can be developed relatively quickly and cost-effectively into a clinical solution for tinnitus. We have plans for the further development of Etanercept in treating of tinnitus, if the proposed clinical trial proves successful. If Etanercept becomes an FDA-approved drug for tinnitus, it would mean a dramatic improvement in treatment options and standard of care. According to tinnitus healthcare experts, such a drug would be quickly adopted and have a multibillion-dollar market.

If the drug is deployed to the battle field (frontline hospital), it could dramatically reduce the incidence of blast- and noise trauma-induced tinnitus. It will not only improve the quality of life for military and Veteran tinnitus sufferers, but it will also save the government hundreds of millions of dollars in disability compensation costs, which is believed to only increase in the coming years.

2.4 Preclinical Data

In addition to acting on cellular inflammatory responses[10], both acute and chronic TNF-α signaling is involved in homeostatic plasticity in the brain[11-14], which is recognized as a potential mechanism underlying tinnitus[15-17] and associated limbic function[18]. Specifically, TNF-α can be activated by sensory deprivation[19], subsequently strengthening excitatory synapses[12-14,20,21] and weakening inhibitory synapses[14,20]: both of which increase neuronal excitability and are implicated in tinnitus etiology[15-17,22-24]. Since TNF-α increases neuronal excitability in response to reduced sensory input[19], it could be a risk factor for tinnitus. Several lines of evidence support this. For example, tinnitus has been considered an auditory analogue of neurological pain due to the comorbidities[25] and similarities between the two conditions[26-28], so it is interesting to note that peripheral nerve injury increases TNF-α expression in the brain, which mediates a central component of neuropathic pain[29,30]. Further evidence demonstrates that after neutralization of TNF-α, abnormal nociceptive CNS activity in the thalamus and limbic system is blocked[31]. In addition, there is circumstantial evidence that TNF-α may be related to tinnitus in humans. For example, Misoprostol, a synthetic prostaglandin E1 analogue that inhibits TNF-α release[32], significantly reduces tinnitus severity and loudness in the clinic[33,34]. By contrast, nonsteroidal anti-inflammatory drugs, known to cause tinnitus at high doses, have been shown to increase TNF-α production[35,36] and reduce neuronal inhibition in the central auditory pathway[37].

To test whether TNF-α is a risk factor for tinnitus in animal models, we examined noise-induced tinnitus in mice with the TNF-α gene knocked out. Our results showed that, after noise exposure, tinnitus occurred in the wildtype but not in TNF-α knockout mice. This demonstrates that TNF-α contributes to the induction of tinnitus.

To test whether TNF-α is sufficient to cause tinnitus, we infused mouse recombinant TNF-α into the right hemisphere of the auditory cortex of normal-hearing wildtype (WT) and TNF-α knockout (KO) mice. Our results indicated that cortical infusion of TNF-α significantly induced tinnitus. Conversely, infusion of mouse albumin did not induce tinnitus behavior.

To test the therapeutic effects of TNF-***α*** blockers, we found that both Etanercept and 3,6'-dithiothalidomide significantly suppressed blast-induced tinnitus (US Patent No. 62/364,600)

Together, those findings suggest an important role for TNF-α in tinnitus and blocking TNF-α suppresses tinnitus.

2.5 Clinical Data

In line with animal studies demonstrating that blocking TNF-α with Etanercept significantly attenuates the cochlear inflammatory response[38,39], a number of human studies have shown therapeutic effects of **Etanercept** on hearing. For example, in one study, 23 patients with bilateral, immune-mediated cochleovestibular disorders or bilateral Meniere's disease were treated with Etanercept for 24 weeks (25 mg twice weekly, by subcutaneous injection). All subjects showed progressive hearing loss within three months prior to the study, but following drug treatment, hearing improved in 33% of the patients[40] or at least stabilized in 87% of patients with pretreatment intractable progressive hearing loss[40]. Another study showed that **Etanercept** improved (7 of 12 patients) or stabilized hearing (4 of 12 patients) of patients with immune-mediated cochleovestibular disorders over a 5-12 month period[41]. Local administration, via transtympanic delivery, of the TNF-α blocker infliximab, once weekly for four weeks, also resulted in hearing improvement[42]. Mechanistically, vascular pathology is a common concept in the pathogenesis of inner ear disorders, including presbycusis, tinnitus, and noise-induced hearing loss. TNF-α, via sphingosine-1-phosphate signaling, may induce a proconstrictive state throughout the cochlear microvasculature, which causes ischemic hearing loss. Thus, treating with **Etanercept** can improve hearing[43].

Human studies also indicate that blocking TNF-***α*** with **Etanercept** suppresses chronic tinnitus by decreasing psychoneuroimmunological stressors. There is a strong linkage between tinnitus and psychological distress[***44-47***]. Tinnitus-related activation of the auditory cortex may trigger a signal to the limbic networks and cause "tinnitus-related distress"[48,49], which manifests by pathological auditory attention to the tinnitus sound; this can lead to increased irritability, insomnia, anxiety, depressive mood, and difficulties with concentration[50,51]. Thus, tinnitus may serve as a chronic stressor that triggers a vicious cycle of tinnitus→stress→exacerbation of tinnitus[***52***]. As stated earlier, proinflammatory cytokines not only act on the immune system but also on the nervous system[11]. Many psychoneuroimmunological studies have demonstrated that psychological distress is associated with immune changes[53-55]. In addition, circulating proinflammatory cytokines have been used as biomarkers for conditions such as post-traumatic stress disorder[56], chronic stress[57,58] or major depression[59]. More specifically, concentrations of TNF-α are

found to be closely associated with tinnitus loudness, total perceived stress, tension, and depression. Furthermore, a negative association exists between TNF-α and a psychometric score "joy"[60]. Importantly, there is an association between tinnitus improvement and a decrease in the concentration of circulating TNF-α[61]. Therefore, pro-inflammatory cytokines may be important in the etiology of tinnitus, and down-regulation of pro-inflammatory cytokines such as TNF-α, an important stress marker[62], may help relieve tinnitus.

It has also been reported that using Misoprostol, a synthetic prostaglandin E1 analogue that inhibits TNF-α release[32], significantly reduces tinnitus severity and loudness in the clinic[33,34]. Additional evidence comes from a study showing that decreased perception of tinnitus, stress, anxious depression, and anger by relaxation training is paralleled by a significant reduction of TNF-α[61]

Very recently, our team collected pilot data from a 72-year old patient who experienced significant improvement in tinnitus and hearing following treatment with the TNF-alpha blocker, Remicade. Etanercept will be used, instead, because it can be more conveniently administered by subcutaneous injection whereas Remicade is usually given by IV infusion together with methotrexate, and is much less expensive than Remicade.

Together, those findings from human studies demonstrate that an important role for TNF-α in tinnitus and hearing loss and blocking TNF-α suppresses tinnitus and improve hearing, via acting on the immune system to stop inflammatory processes and resetting the maladaptive neural plasticity that underlies both hearing loss and tinnitus.

2.6 Dosage and frequency

The administration of Etanercept generally follows the guidelines in the drug label for Etanercept that has been published by Amgen. The supporting human studies discussed in Section 2.5 for treating hearing loss and tinnitus also follow this study dosage and frequency. Etanercept 50 mg will be administered once per week for twelve consecutive weeks. Missed study drug can be administered within 48 hours of scheduled dosing day based on randomization. If subject is unable to complete dosing visit with 48 hours the subject should defer dosing until next scheduled dosing visit.

See details at the following link for package insert:
https://pi.amgen.com/~/media/amgen/repositorysites/pi-amgen-com/enbrel/enbrel_pi.ashx

3.0 DRUG

3.1 Drug Acquisition, Packaging, Labeling and Storage

Packaging
Etanercept lyophilized powder in a multiple-dose vial will be used. Consistent with Amgen's drug labeling.

Etanercept for Injection is supplied as lyophilized powder for reconstitution in a multiple-dose vial. Each vial is supplied in a carton containing four dose trays. Each dose tray contains one 25 mg vial of etanercept lyophilized powder, one diluent syringe (1 mL Sterile Bacteriostatic Water for Injection, USP, containing 0.9% benzyl alcohol), one 27-gauge ½-inch needle, one vial adapter, and one plunger. Each carton contains four "Mixing Date:" stickers.

Reconstitution

Etanercept lyophilized powder should be reconstituted aseptically with 1 mL of the supplied Sterile Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol), giving a solution of 1 mL containing 25 mg of Etanercept.

For a more comfortable injection, leave the Enbrel dose tray at room temperature for about 15 to 30 minutes before injecting.

There are two methods for preparing the Enbrel solution, the vial adapter method should NOT be used if the vial is being used more than once.

Vial Adapter Method:

1. Remove the pink plastic cap from the Enbrel vial. Do not remove the gray stopper or silver metal ring around the top of the Enbrel vial.
2. Place the Enbrel vial on your flat work surface or turn your dose tray upside down and place your Enbrel vial in the round space marked "V". Use one alcohol swab to clean the gray stopper on the Enbrel vial. Do not touch the gray stopper with your hands.
3. Open the wrapper that contains the 27-gauge needle by peeling apart the tabs and set the needle aside for later use.
4. Open the wrapper that contains the vial adapter by peeling apart the tabs and set the vial adapter aside for later use. Do not touch the vial adapter's twist-on end or the spike inside.
5. Slide the plunger into the flange end of the syringe.
6. Attach the plunger to the gray rubber stopper in the syringe by turning the plunger clockwise until you feel a slight resistance.
7. Remove the twist-off cap from the prefilled diluent syringe by turning counter-clockwise. Do not bump or touch the plunger. Doing so could cause the liquid to leak out. You may see a drop of liquid when removing the cap. This is normal. Place the cap on your flat work surface. Do not touch the syringe tip.
8. Once the twist-off cap is removed, pick up the vial adapter with your free hand. Twist the vial adapter onto the syringe, turning clockwise until you feel a slight resistance. Do not over-tighten.
9. Hold the Enbrel vial upright on your flat work surface. Grasp the sides of the vial adapter and place it over the top of the Enbrel vial. Do not bump or touch the plunger. Doing so could cause the liquid to leak out. Insert the vial adapter into the gray stopper on the Enbrel vial. The plastic spike inside the vial adapter should puncture the gray stopper. The vial adapter should fit snugly.
10. Hold the Enbrel vial upright on your flat work surface and push the plunger down until all the liquid from the syringe is in the Enbrel vial. You may see foaming (bubbles) in the vial. This is normal.

11. Gently swirl the Enbrel vial in a circular motion to dissolve the powder. If you used the dose tray to hold your Enbrel vial, take the vial (with the vial adapter and syringe still attached) out of the dose tray, and gently swirl the vial in a circular motion to dissolve the powder. Do not shake. Wait until all the powder dissolves (usually less than 10 minutes). The solution should be clear and colorless. After the powder has completely dissolved, foam (bubbles) may still be present. This is normal. Do not inject the solution if it is discolored, contains lumps, flakes, or particles.
12. Turn the Enbrel vial upside down. Remove the entire volume (1 mL), unless otherwise instructed by your healthcare provider. Be careful not to pull the plunger completely out of the syringe. Some white foam may remain in the Enbrel vial. This is normal.
13. Check for air bubbles in the syringe. Gently tap the syringe to make any air bubbles rise to the top of the syringe. Slowly push the plunger up to remove the air bubbles. If you push solution back into the vial, slowly pull back on the plunger to again draw the correct amount of solution back into the syringe.
14. Remove the syringe from the vial adapter, by holding the vial adapter with one hand and turning the syringe counter-clockwise with your other hand. Do not touch or bump the plunger. Place the Enbrel vial with the vial adapter on your flat work surface.
15. Continue to hold the barrel of the syringe. With your free hand, twist the 27-gauge needle onto the tip of the syringe until it fits snugly. Do not remove the needle cover from the syringe. Place the syringe on your flat work surface until you are ready to inject Enbrel.

Amgen Package Insert: https://www.pi.amgen.com/~/media/amgen/repositorysites/pi-amgen-com/enbrel/enbrel_muvifu.pdf

A vial adapter is supplied for use when reconstituting the lyophilized powder. However, the vial adapter should not be used if multiple doses are going to be withdrawn from the vial. If the vial will be used for multiple doses, a 25-gauge needle should be used for reconstituting and withdrawing Etanercept, and the supplied "Mixing Date:" sticker should be attached to the vial and the date of reconstitution entered. Reconstituted solution must be refrigerated at 36°F to 46°F (2°C to 8°C) and used within 14 days. Discard reconstituted solution after 14 days because product stability and sterility cannot be assured after 14 days. DO NOT store reconstituted Etanercept solution at room temperature.

Administration
Etanercept will be used under the guidance and supervision of a physician.
Study drug will be administered via subcutaneous injection in the outer area of the upper arm. Optional sites for administration include the front of the middle thighs and the abdomen area (except for the 2-inch area around the naval). It is best to rotate the injection site for each injection. Do not inject into areas where the skin is tender, bruised, red, or hard. Avoid areas with scars or stretch marks. If a subject has psoriasis, do not inject into any raised, thick, red, or scaly patches or lesions.

Storage
Etanercept multiple-dose vial should be refrigerated at 36°F to 46°F (2°C to 8°C) and in the original carton to protect from light or physical damage.

If needed, individual dose trays containing Etanercept multiple-dose vial and diluent syringe can be stored at room temperature at 68°F to 77°F (20°C to 25°C) for a maximum single period of 14 days, with protection from light, sources of heat, and humidity. Once the dose tray has reached room temperature, do not put it back into the refrigerator. The multiple-dose vial that has been stored at room temperature for 14 days should then be discarded.

Mixed (reconstituted) Etanercept should be used right away or kept in the refrigerator at 36°F to 46°F (2°C to 8°C) for up to 14 days.

Do not store Etanercept in extreme heat or cold. DO NOT FREEZE. Keep out of the reach of children.

Do not use Etanercept beyond the expiration date stamped on the dose tray. DO NOT SHAKE

3.2 Drug Shipment Accountability and Destruction

Sterile investigational study drug Etanercept and saline placebo will be shipped by the lead site Clinical Research Center Investigational Pharmacy to all participating sites for accurate drug accountability.

The investigative site will acknowledge receipt of the products by returning "Receipt of Investigational Product Supplies" to the lead site Investigational Pharmacist. Any supplies damaged in transit will be replaced. A drug and saline placebo accountability log will be maintained by each site's unblinded pharmacist for recording all study products administered to study subjects.

The designated unblinded pharmacist at the study site will maintain adequate records regarding the receipt and disposition of all study drug shipped to the site. Records must include the following: dates of receipt; lot numbers; quantities received and dispensed; time of drug reconstitution and the identification number of each subject. A drug temperature log will also be maintained to document that the drug was stored under specified conditions.

Unused drug product vials will be returned to the lead site investigational pharmacy at the end of the study. After proper documentation in the Drug Accountability Log and approval by the site pharmacist and study monitor, used vials of drug product will be destroyed according to site pharmacy destruction protocol.

If any vials of Etanercept and/or diluent syringe are found to be defective, opened or in abnormal color before use, they are not to be destroyed, but must be returned to the lead site investigational pharmacy after appropriate notations are made in the logs.

3.3 Drug Randomization

Study drug will be block randomized by site 1:1placebo to drug. The site-specific randomization code will be given to the unblinded pharmacist who will use the next consecutive randomization for each subject randomized at a site.

4.0 SUBJECT ELIGIBILITY

All procedures for recruitment, informed consent, and conduct of the study will adhere to the requirements of the Institutional Review Boards at all sites. Participants will be adults who experience tinnitus associated with blast or loud noise exposure. Subjects will be recruited across seven study sites with established otolaryngology and/or audiology clinics, which will ensure adequate access to potential volunteers for this study. Attempts will be made to sample representative numbers of male and female subjects, as well as to recruit subjects in the same proportions of ethnicity as are found in the general population of study sites. However, the study population will be dictated by the demographic mix of tinnitus patients who volunteer to participate. Military personnel, Veterans, and civilians will be recruited for the study. Non-military and non-Veteran civilians will be included because this is necessary to obtain an adequate number of subjects who meet the inclusion/exclusion criteria.

4.1 Inclusion Criteria

To be considered eligible for inclusion, subjects must meet **all** of the following criteria:

1) Tinnitus associated with blast- or noise-exposure of at least a moderate severity as defined by a score of >25 points or higher on the Tinnitus Functional Index (TFI) questionnaire1, and/or a self-rated visual numeric score (VNS) of at least 5 out of 10 for tinnitus loudness.
2) Able to provide written informed consent.
3) Age/Gender: Minimum 18 years of age at the time of enrollment.
4) Other concurrent treatments: A four-week washout from any other tinnitus treatment or management program is required prior to entering this study.
5) Psychological status: Stable enough to complete this study per the opinion of the research team.
6) Hearing function: All degrees of hearing function can be included, recognizing that individuals with profound, bilateral hearing losses will not be able to perform tinnitus evaluations and hearing tests but will be able to rate subjective tinnitus loudness, annoyance and impact on life. This is an important sub-population because of the challenges in treating them with acoustic therapy and the need for a medical intervention.
7) Additional tinnitus characteristics:
    a) Tinnitus history: Onset associated with blast- and/or noise exposure. Subjects will have either recent blast or noise exposure, defined as exposure less than six months ago at time of enrollment, or historical exposure, defined as exposure 6 months or longer ago at time of enrollment.

b) Stability: Constant (not pulsatile, intermittent, varying to a high degree in loudness or changing in location of perception). Fluctuating tinnitus reduces the reliability of test-retest measures for loudness.

c) Location of tinnitus perception: Unrestricted. Tinnitus may be unilateral, bilateral, or perceived in the head.

4.2 Exclusion Criteria

A subject will be **ineligible** for this study if **any one** of the following criteria is met:

1) History or evidence of significant brain malformation or neoplasm, cerebral vascular events (such as strokes), neurodegenerative disorders affecting the brain (such as Parkinson's disease, ALS, Huntington's disease or Multiple sclerosis), or prior brain surgery.
2) History of seizures or epileptic activity.
3) Subjects with cardiac pace makers, other electronic implants (including cochlear implants), or intracranial or intraocular metallic particles.
4) Subjects who currently have an active infection, including tuberculosis and chicken pox.
5) Diagnosis of active neurologic disease, auto-immune disease, a weak immune system, diabetes, HIV, hepatitis B, or current or past heart failure.
6) Ongoing treatment with one of the following contraindicated medications: abatacept, cyclophosphamide or sulfasalazine.
7) Subjects who cannot communicate reliably with research team members or who are not likely to cope with the requirements of the trial.
8) Subjects who have participated in a drug clinical trial within the last 30 days before the start of this one.
9) Current substance abuse (defined as a score of 2 or greater on the CAGE Substance Abuse Screening Tool)
10) Pregnancy or planned pregnancy during the study.
11) Women who are lactating or are of child-bearing-age without use of contraception.
12) Participation in greater than two previous clinical drug-trials for tinnitus.
13) MMSE score < 24

5.0 STUDY DURATION

Baseline assessments will be conducted up 14 days prior to the start of treatment, and outcome measures will be made on the same day of treatment at 1, 4, 8, and 12 weeks during the 12-week treatment period. Follow-up evaluations will be conducted at 4, 8, 12, and 24 weeks after the last treatment session, this will be the same as study schedule week 16, 20, 24 and 36 respectively. The subject participation in the study will be approximately 10 months. The study aims to recruit 310 subjects over 3-4 years.

6.0 TRIAL DESIGN

6.1 Design of Trial

This is a multi-center, prospective, randomized, double blinded, saline placebo-controlled study of Etanercept administered subcutaneously. For mechanistic studies, a subset of 40 subjects will undergo imaging including resting-state fMRI, DTI, and SWI at pre-treatment, 12 weeks during treatment, and 24 weeks after the last treatment, this will be the same as study schedule week 36. Blood serum tests will be administered on another subset of 60 subjects (30 each at a VA and civilian site) to monitor TNF-α concentration at pre-treatment (baseline), 1, and 12 weeks during treatment.

6.1.1 Assignment of Screening Number

Subjects who provide informed consent to participate will receive a screening number. Each subject will receive a 6 digit alphanumeric number that will be used to identify the subject and site for all procedures that occur prior to randomization or treatment allocation (example 01- S001). Each subject will be assigned only one screening number. Screening numbers must not be re –used for different subjects. Subject screened multiple times will use the original screening number at their initial visit.

6.1.2 Assignment of Treatment/Randomization Number

Subjects who meet all eligibility criteria will be allocated, by non-random assignment, and will receive a treatment/randomization number. Each subject will receive a 5 digit alphanumeric number that will be used to identify the subject and site for all procedures occurring after treatment allocation/randomization (example 01-001). Treatment/randomization numbers must not be re-used for different subjects. A subject cannot be assigned with more than one treatment/randomization number.

6.2 Study Schedule for Tinnitus and Hearing Loss and Related Outcome Measurements

After signing the informed consent and HIPAA authorization forms, subjects will enter the screening phase of the clinical trial. Subjects will then undergo baseline evaluation to determine eligibility for the study and establish subject's baseline scores and responses. Baseline procedures can be performed over multiple visits within 14 days. Subjects who do not meet the eligibility criteria are considered screening failures. Subjects who meet all eligibility criteria will be enrolled and randomized to receive either Etanercept (50 mg) or a saline solution placebo administered once per week for twelve consecutive weeks Etanercept or placebo will be given under the guidance of participating clinician, nurse or qualified medical staff.

Audiometric and Tinnitus questionnaires administrations will be conducted at weeks 1, 4, 8, and 12 weeks during the 12-week treatment period. The post-drug follow-up evaluations

will be conducted at study week 16, 20, 24 and 36 as detailed in the schedule of events table, page 8 of the protocol.

MRI Sub-Study

A subset of 40 subjects will undergo MRI imaging at pre-treatment, 12 weeks during treatment, and 24 weeks after the last treatment this will be the same as study schedule week 36. This sub-study will only be conducted at sites located in Michigan.

TNF-α Sub-Study

Blood serum tests will be administered on another subset of 60 subjects to monitor TNF-α concentration at pre-treatment, 1, and 12 weeks during treatment. This sub-study will only be conducted at sites located in Michigan.

6.3 Screening for Tinnitus, Hearing and Measurements

To identify eligible candidates, study personnel may screen potential participants via telephone. If candidates fail to meet the selection criteria during the phone screening, they can be re-screened at a later date at the discretion of staff. When a candidate is re-screened, they will be contacted via the phone and re-asked the phone screening questions following the IRB approved script. If they meet the criteria to be invited for the in-person screening, they will be scheduled at that time. Total time to complete all questionnaires will be approximately 90 minutes.

Participant Questionnaires

1. Visual Numerical Scale (VNS) for self-rated tinnitus loudness. A score of at least 5 out of 10 for tinnitus loudness is required for participation in the study
2. Mini-Mental State Examination (MMSE)[63]. A minimum MMSE score of 24 will be required for participation in the study, to identify and exclude subjects with dementia or other forms of cognitive impairment
3. Tinnitus History Questionnaire
4. Medical History Questionnaire
5. CAGE Substance Abuse Screening Tool
6. Blast Questionnaire
7. Tinnitus Functional Index (TFI); A score of ≥ 25 points is required for participation in the study
8. Tinnitus Primary Function Questionnaire (TPF)
9. Hospital Anxiety and Depression Scale
10. Short-Form Health Survey 12-items
11. Speech, Spatial and Qualities of Hearing questionnaire

VNS Scale

VNS for self-rated tinnitus loudness: On the scale below, please draw a vertical line to indicate the loudness of your tinnitus at this moment

0	1	2	3	4	5	6	7	8	9	10
NO TINNITUS										**VERY LOUD**

Audiologic Testing

First, a hearing specialist will look into subjects' ears with an ear light to check for wax or other substances. In addition, he/she will check for obvious conditions that could interfere with the tests to be conducted. Second, foam earphones (similar to small insert stereo phones) will be placed in the ear canals. Subjects will be asked to listen and respond to tones of differing pitches and at varying levels of loudness. Third, subjects will be asked to repeat a series of words of varying loudness. During the audiologic assessment, the audiologist will insert a probe tip made of soft, pliable plastic into the ear canal and subjects will feel a slight change in pressure and hear a soft tone. All of these procedures are similar to tests that normally are used to evaluate hearing in an audiology clinic. These hearing tests are expected to take approximately 45 minutes to complete.

The audiologist will administer the following baseline assessments according to guidelines established by the American Speech-Language-Hearing Association (ASHA). The testing will take approximately 30-45 minutes:

1. Otoscopy
2. Pure tone air conduction (AC) testing
3. Pure tone bone conduction (BC) testing
4. Speech recognition threshold (SRT)
5. Tympanometry
6. Word recognition testing

Otoscopy

This test will be conducted at Baseline and during the treatment phase at weeks 1, 4, 8, and 12 and also during the post-treatment follow-up phase at 16, 20, 24 and 36 weeks

Pure tone air conduction (AC) testing

This test will be conducted at Baseline and during the treatment phase at weeks 1, 4, 8, and 12 and also during the post-treatment follow-up phase at 16, 20, 24 and 36 weeks

Pure tone bone conduction (BC) testing

This test will be conducted at Baseline and at future visits only if greater than 10 dB change in AC threshold are observed at one or more frequencies.

Speech recognition threshold (SRT)
This test will be conducted at Baseline and during the treatment phase at weeks 1, 4, 8, and 12 and also during the post-treatment follow-up phase at 16, 20, 24 and 36 weeks.

Tympanometry
This test will be conducted at Baseline and at future visits only if there is an air bone gap of 15 dB or greater at two adjacent frequencies, or 20 dB or greater at any one frequency.

Word recognition testing
This test will be conducted at Baseline, Week 12, Week 24 and Week 36.

Tinnitus testing includes:
1. Tinnitus loudness matching (1 kHz loudness-matching[64])
2. Minimum Masking Levels (MMLs)[64,65].

Tinnitus loudness matching. This test involves going into a sound booth and having foam earphones inserted into ear canals by the audiologist. The audiologist will then play a 1 kHz pure-tone presented to the ear contralateral to the side of the tinnitus perception. In cases of bilateral tinnitus that does not lateralize to one side or is perceived "in the head" the contralateral ear will be selected based on whichever ear has the better pure-tone hearing threshold at 1 kHz. The goal is to find the intensity level of a 1 kHz pure-tone that best matches the loudness of his/her tinnitus. This test will be conducted at Baseline and during the treatment phase at weeks 1, 4, 8, and 12 and also during the post-treatment follow-up phase at 16, 20, 24 and 36 weeks.

Minimum masking levels (MMLs): After obtaining the 1 kHz tinnitus loudness match, the audiologist will obtain hearing thresholds for a band of noise for each ear separately. Next, the noise will be presented at the same sensation level binaurally until the participant states the tinnitus is completely or partially masked. The goal is for a subject to tell the audiologist the softest intensity level of noise that will cover (or "mask") the sound of the subject's tinnitus. This test will be conducted at Baseline and during the treatment phase at weeks 1, 4, 8, and 12 and also during the post-treatment follow-up phase at 16, 20, 24 and 36 weeks.

<u>Physical exam</u>
A physician or qualified health care professional will measure vital signs (height, weight, blood pressure, heart rate, temperature and respiration rate) discuss medical, medication and tinnitus history. A complete physical exam will be done, including ear, nose and throat.

<u>TB test</u>
A TB test (either skin test or QuantiFERON blood test) will be performed at baseline and at the end of the study.

<u>Safety Laboratory Testing</u>
A CBC with differential and an electrolyte panel will be conducted at Baseline, 4 weeks and 12 weeks during the treatment phase.

6.4 TNF-α Sub-Study

To monitor TNF-α concentration changes during the course of Etanercept treatment, blood serum tests will be administered on a subset of 60 subjects to monitor TNF-α concentration at pre-treatment, 1, and 12 weeks during treatment. All assays will be performed in ARUP laboratories. Thirty subjects (15 subjects with active Etanercept treatment and 15 placebo controls) will come from each a VA and civilian site. This secondary hypothesis is not powered; rather, the data will be collected for secondary feasibility analysis only. This sub-study is only being conducted at Michigan sites.

Blood collection and serum processing. The serum TNF-alpha test is not affected by eating therefore fasting is not required. TNF-alpha levels do not fluctuate significantly throughout the day and blood can be drawn at any time. The blood draw can cause some anxiety, which can be severe for some people. Questionnaires and assessments should be completed prior to the blood draw.

Venous blood will be collected using blood collection plasma tubes (BD Vacutainer® PST, cat.# MON 36792810). The blood will be allowed to coagulate for 30 min at room temperature, and then centrifuged in the collection tubes. The sera will be pipetted into 200 ml aliquots, labeled and frozen at -80°C. All samples, as well as the psychometric scores and other data will be coded with a unique study identification number.

6.5 MRI Sub-Study Procedures and Measurements

MRI Scanning procedures

40 subset of subjects who meet the inclusion and exclusion criteria and are recruited with history of tinnitus (< 6 months onset) will be recruited from Michigan sites from both military and civilian population. Each subject will be scanned at baseline, 12 and 24 weeks. Each MRI scan will take less than 30 min. Once a potential subject has been identified, the study coordinator will escort the subject to the MR Research Facility for his/her baseline scan. Each subject is required to fill out the MR safety screening sheet before coming to the MR Research facility to ensure that it is safe for the subject to have an MRI. This form will be reviewed by the MR Technologist to make sure the subject has no contraindications for an MRI. Urine pregnancy test will be performed prior to each MRI for all female subjects.

MR Scanning protocol.

Magnetic Resonance Research Facility 3T (MAGNETOM® Verio)								
MRRC review committee - Magnet time application								
Date: 04/28/2018								
Study Name: Clinical Trial of Etanercept (TNF-α Blocker) for Treatment of Blast-Induced Tinnitus								
	Batch 1		**Batch 2**			**Batch 3**		
Sequence Name	**T1 - MPRAGE**	**3D Flair**	**new_B1 SWI**	**new_B2 SWI**	**new_B3 SWI**	**DTI**	**EPI Resting**	**EPI_Pasl_2D**
Orientation	Sagital	Sagittal	Axial	Axial	Axial	Axial	Axial	Axial
TR (ms)	1680	6000	25	25	20	3500	2600	3500
TE (ms)	3.43	364	7.5 , 17.5	8.75 , 18.75	2.5 , 12.5	87	30	22.76
TI (ms)	900	2200	N/A	N/A	N/A	N/A	N/A	-
FA (degree)	9	N/A	6	24	12	N/A	90	180
Base Resolution	384	384	384	384	384	100	64	64
FOV phase (%)	100%	100%	75%	75%	75%	100%	100%	81.3%
Matrix size	384 x 384	384 x 384	144 x 384	144 x 384	144 x 384	200 x 200	200 x 200	64 x 64
FOVphase x FOVread	256 x 256	258 x 256	192 x 256	192 x 256	192 x 256	100 x 100	100 x 100	182 x 224
TH (mm)	1.3	1	2	2	2	2	3.5	3.5
No of Slices	160	160	80	80	80	84	33	32
Voxel size (mm3)	0.7x0.7x1.4	0.7x0.7x1.4	1.4x0.7x2	1.4x0.7x2	0.7x0.7x2	2 x 2 x 2	2x2x4	3.5x3.5x3.5
#DTI dir	N/A	N/A	N/A	N/A	N/A	96 (free mode)	N/A	-
Phase resolution	100%	101%	50%	50%	100%	100%	100%	100%
Slice Resolution	100%	100%	100%	100%	100%	N/A	N/A	100%
Accel. factor PE	2	2	2	2	2	2	2	2
Ref. lines PE	24	24	24	24	24	24	24	24
Multi band Acc Factor	N/A	N/A	n/A	n/A	n/A	3	N/A	N/A
BW (Hz/Px)	180	781	240,240	240,240	240,240	1724	2368	2004
Flow Comp	Slice	N/A	yes,yes	yes,yes	yes,yes	N/A	N/A	N/A
Scanner Acquisition Time (sec)	**0:04:07**	**0:03:33**	**0:03:04**	**0:03:04**	**0:05:29**	**0:06:22**	**0:05:13**	**03:34**
Batch Time	**0:09:28**		**0:03:04**			**0:15:09**		

MRI Measurements

The following outcome measures will be evaluated:

1) T1MPRAGE will be used for volumetric segmentation to measure the cortical thickness, volume and curvature of different structures associated with tinnitus.
2) SWI: SWI data will be processed to determine venous oxygen saturation, which is the main determinant of the measured susceptibility of blood. Additionally, it is very sensitive to micro hemorrhages of the brain after trauma. The micro hemorrhages will be estimated in a semi-automated method to count the overall lesion load and number. In addition, the lesion load and numbers will also be quantified in each major brain regions.
3) DTI: A whole brain white matter analysis will be performed to measure the global white matter injury. In addition, a semi-automated analysis will be performed on 40 white matter brain regions to identify which white matter structure is more susceptible for

injury. Hence, it is important to study the relationship between the macrostructural measure, ie, volumetric measurement, derived from structural T1-weighted imaging and the microstructural measures of WM integrity indices, such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), using diffusion tensor imaging (DTI) in the two groups.

4) RS-fMRI: Signal change, regional homogeneity (ReHo), and amplitude of low frequency fluctuation (ALFE) will be retrieved and calculated from blood oxygen level dependent (BOLD) signals respectively. Both global and ROI analysis of BOLD response to the cognitive task will be applied to examine cortical activation patterns in both etanercept treated (Group 1) and placebo treated tinnitus patients.
5) pCASL: This is a whole brain, gray matter-biased technique. Pseudocontinuous arterial spin labeling (pCASL) will be used to assess the regional cerebral blood flow (rCBF) of the brain in a non-invasive way. pCASL is a non-invasive MR pulse sequence that can measure the CBF of the brain.

6.6 Early Withdrawal from Treatment & Continued Data Collection (Intent to Treat)

Subjects have the option to withdraw their participation at any time during this clinical trial and investigators have the right to terminate participation of any subject. The primary consideration in any determination to discontinue a subject's participation must be the health and welfare of the subject.
As this research is an "intent-to-treat" study design, it is important to point out the distinction between withdrawal from treatment and withdrawal from the study. With intent-to-treat study designs, subjects who are withdrawn from treatment continue to be followed and have data collected at all scheduled outcome evaluations. This method reduces introducing bias into the estimate of treatment efficacy. All subjects will be monitored for possible adverse events.

Week 12 assessments will be completed if a participant withdraws during the treatment period.

Week 36 assessments will be completed if a participant withdraws during the post treatment period

7.0 SCHEDULE OF VISITS

It is preferable to complete all testing and questionnaires prior to blood draw and MRI.

7.1 Baseline/ Visit 1 (-14 days) includes:

- Informed consent and explaining of study
- Verification of inclusion/exclusion criteria
- Physical exam
- Vital Signs
- Previous medications
- Pregnancy test

- Tuberculosis test
- CBC with diff and an electrolyte panel
- Tinnitus history questionnaire
- Medical history questionnaire
- CAGE Substance Abuse Screening Tool
- Blast questionnaire
- Mini Mental State Examination (MMSE)
- Speech Spatial & Qualities of Hearing (SSQ-12)
- Visual Numeric Scale (VNS)
- Tinnitus Functional Index (TFI)
- Tinnitus Primary Function (TFP) questionnaire
- Hospital Anxiety and Depression Scale (HADS)
- Short Form Health Survey 12 items
- Audiologic testing
  - Pure Tone Air Conduction (AC) testing
  - Pure Tone Bone Conduction (BC) testing
  - Speech Reception Threshold (SRT)
  - Tympanometry
  - Word Recognition Testing
  - Tinnitus loudness matching test
  - Minimum Masking Levels (MML) test
- Blood serum (drawn only on the sub study participants)
- MRI testing (conducted only on the sub study participants)
- AE/SAE
- Review of concomitant medications

7.2 Visit 2 (Week 1) (+/- 2 days) includes assessments and reporting of

- Vital signs
- Visual Numeric Scale (VNS)
- Audiologic testing
  - Pure Tone Air Conduction (AC) testing
  - Pure Tone Bone Conduction (BC) testing (as required based on dB change in AC threshold-section 6.3).
  - Speech Reception Threshold (SRT)
  - Tympanometry (as required based on dB change in bone gap-section 6.3).
  - Tinnitus loudness matching test
  - Minimum Masking Levels (MML) test
- Blood serum will be drawn only on the sub study participants
- AE/SAE
- Review of concomitant medications
- Administer study drug

7.3 Visit 3 & 4 (Week 2, Week 3) (+/- 2 days) includes

- Vital signs
- Review of concomitant medications
- AE/SAE
- Administer study drug

7.4 Visit 5 (Week 4) (+/- 2 days) includes

- Vital signs
- Visual Numeric Scale (VNS)
- Tinnitus Functional Index (TFI)
- Audiologic testing
  - Pure Tone Air Conduction (AC) testing
  - Pure Tone Bone Conduction (BC) testing (as required based on dB change in AC threshold-section 6.3).
  - Speech Reception Threshold (SRT)
  - Tympanometry (as required based on dB change in bone gap-section 6.3).
  - Tinnitus loudness matching test
  - Minimum Masking Levels (MML) test
- Review of concomitant medications
- AE/SAE
- CBC with diff and an electrolyte panel
- Administer study drug

7.5 Visit 6, 7 & 8 (Week 5, Week 6, Week 7) (+/- 2 days) includes

- Vital signs
- Review of concomitant medications
- AE/SAE
- Administer study drug

7.6 Visit 9 (Week 8) (+/- 2 days) includes

- Vital signs
- Visual Numeric Scale (VNS)
- Tinnitus Functional Index (TFI)
- Audiologic testing
  - Pure Tone Air Conduction (AC) testing
  - Pure Tone Bone Conduction (BC) testing (as required based on dB change in AC threshold-section 6.3).
  - Speech Reception Threshold (SRT)
  - Tympanometry (as required based on dB change in bone gap-section 6.3).
  - Tinnitus loudness matching test
  - Minimum Masking Levels (MML) test
- Blood serum will be drawn only on the sub study participants
- AE/SAE
- Review of concomitant medications
- Administer study drug

7.7 Visit 10, 11 & 12 (Week 9, Week 10, Week 11) (+/- 2 days) includes

- Vital signs
- Review of concomitant medications
- AE/SAE
- Administer study drug

7.8 Visit 13 (Week 12) (+/- 2 days) includes

- Vital signs
- Visual Numeric Scale (VNS)
- Speech Spatial & Qualities of Hearing (SSQ-12)
- Tinnitus Functional Index (TFI)
- Tinnitus Primary Function (TFP) questionnaire
- Hospital Anxiety and Depression Scale (HADS)
- Short Form Health Survey 12 items
- Audiologic testing
  - Pure Tone Air Conduction (AC) testing
  - Pure Tone Bone Conduction (BC) testing (as required based on dB change in AC threshold-section 6.3).
  - Speech Reception Threshold (SRT)
  - Tympanometry (as required based on dB change in bone gap-section 6.3).
  - Word Recognition Testing
  - Tinnitus loudness matching test
  - Minimum Masking Levels (MML) test
- AE/SAE
- Review of concomitant medications
- CBC with diff and an electrolyte panel
- Administer study drug
- MRI testing will be conducted only on the sub study participants

7.9 Visit 14 & 15 (Week 16, Week 20) (+/- 2 days) includes

- Vital signs
- Visual Numeric Scale (VNS)
- Tinnitus Functional Index (TFI)
- Audiologic testing
  - Pure Tone Air Conduction (AC) testing
  - Pure Tone Bone Conduction (BC) testing (as required based on dB change in AC threshold-section 6.3).
  - Speech Reception Threshold (SRT)
  - Tympanometry (as required based on dB change in bone gap-section 6.3).
  - Tinnitus loudness matching test
  - Minimum Masking Levels (MML) test
- AE/SAE
- Review of concomitant medications

7.10 Visit 16 (Week 24) (+/- 2 days) includes

- Vital signs
- Speech Spatial & Qualities of Hearing (SSQ-12)
- Visual Numeric Scale (VNS)
- Tinnitus Functional Index (TFI)
- Tinnitus Primary Function (TFP) questionnaire
- Hospital Anxiety and Depression Scale (HADS)
- Short Form Health Survey 12 items
- Audiologic testing

- Pure Tone Air Conduction (AC) testing
- Pure Tone Bone Conduction (BC) testing (as required based on dB change in AC threshold-section 6.3).
- Speech Reception Threshold (SRT)
- Tympanometry (as required based on dB change in bone gap-section 6.3).
- Tinnitus loudness matching test
- Minimum Masking Levels (MML) test

- AE/SAE
- Review of concomitant medications
- MRI testing will be conducted only on the sub study participants

7.11 Visit 17 (Week 36) (+/- 2 days) includes

- Vital signs
- TB Test
- Tinnitus History Questionnaire
- Speech Spatial & Qualities of Hearing (SSQ-12)
- Visual Numeric Scale (VNS)
- Tinnitus Functional Index (TFI)
- Tinnitus Primary Function (TFP) questionnaire
- Hospital Anxiety and Depression Scale (HADS)
- Short Form Health Survey 12 items
- Audiologic testing
  - Pure Tone Air Conduction (AC) testing
  - Pure Tone Bone Conduction (BC) testing (as required based on dB change in AC threshold-section 6.3).
  - Speech Reception Threshold (SRT)
  - Tympanometry (as required based on dB change in bone gap-section 6.3).
  - Word Recognition Testing
  - Tinnitus loudness matching test
  - Minimum Masking Levels (MML) test
- AE/SAE
- Review of concomitant medications

8.0 SUBJECT / TRIAL COMPLETION CRITERIA

8.1 Termination Criteria

All Serious Adverse Events will be reviewed by the Medical Monitor and reported to the Data Safety Monitoring Committee.

The lead site has the right to stop the study prior to meeting enrollment goals. The PIs, after discussion with the lead site and medical monitor, have the right to stop the study at the site for safety reasons at any time.

If a decision is made to stop the study, investigators are required to contact all active study participants. All data collection forms must be completed up to the point of study termination.

8.2 Early Withdrawal from Study

See section 6.9

8.3 Early Termination of Study

The PI/Site PIs reserve the right to terminate this study at any time and for any reason. Any subject who has been drug-administered will be followed to a logical endpoint so that his/her safety is not compromised. Subjects terminating before completion of the study will be asked to undergo end-of-study evaluations.

9.0 SAFETY ASSESSMENTS

The following assessments will be done at the study visits to assess safety throughout the study:

- Medical history including inventory of currently prescribed medications
- Physical exam including otoscopy and vital signs (temperature, heart rate and blood pressure [systolic, diastolic])
- Audiological evaluation
- Tinnitus evaluation
- Psychological evaluation (interrogatory and physical examination; specific focus on depression and other psychiatric pathologies)
- CBC with diff and an electrolyte panel
- Adverse event assessment

9.1 Adverse Event (AE) Management

In the event of an untoward medical occurrence (eg, hospitalization for elective surgery if planned before the start of the study, admissions for social reasons or convenience), and anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen are not considered an AEs.

Cases of pregnancy that occur during maternal or paternal exposure to investigational product are to be reported within 24 hours of Investigator/site awareness.

Adverse events should be recorded from the time of signed consent.

9.1.1 Adverse Events Definitions:

a. Adverse Event:
Any untoward occurrence in a patient or subject administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment. An AE can be a sign (including abnormal lab findings), symptom, disease or test result.

b. Serious Adverse Event (SAE):
Any untoward medical experience or reaction that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or a congenital anomaly or birth defect. Other important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above may also be classified as serious adverse events.

c. Significant but Non-serious Adverse Event:
Any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the study drug, whether or not considered related to the study drug, including (but not limited to) those events resulting from use as stipulated in the protocol and that lead to an intervention by a health care professional. Signs and symptoms associated with adverse events already identified and reported need not be reported separately (e.g. fever with sepsis or jaundice with liver failure). Laboratory or radiographic abnormalities that, in the opinion of the investigators, are not part of a general clinical deterioration, or have been previously reported as part of another adverse event, do not require separate reporting.

d. Adverse drug reactions. Events that may be related to the study drug are called adverse drug reactions (ADR).

9.1.2 Severity of Adverse Events:
Adverse events will be categorized into four different levels of severity (mild, moderate, severe and life threatening)

These categories are defined as follow:

- Mild - transient and easily tolerated.
- Moderate - results in a modification or interruption of the subject's usual activities or care and may require discontinuation of the clinical product.
- Severe - results in considerable interference with the subject's usual activities or care and may require discontinuation of study drug.
- Life Threatening - handled as SAE.

9.1.3 Relationship to Study Drug:
The investigator/site physician will assess the relationship of the adverse event to the study drug using the following definitions:

Probable: An adverse event has a strong temporal relationship to the study drug or recurs on re-challenge and another etiology is unlikely or significantly less likely.

Possible: An adverse event has a strong temporal relationship to the study drug and an alternative etiology is equally or less likely compared to the potential relationship to study drug.

Probably Not: An adverse event has little or no temporal relationship to the study drug and/or a more likely alternative etiology exists.

Not Related: An adverse event is due to an underlying or concurrent illness and is not related to the study drug.

Note: If a serious adverse event is judged to be "possible", "probably not" or "not related," an alternative possible etiology must be given by the investigator.

9.1.4. Adverse Event Collection and Reporting

The AE/SAE reporting period for participants is from the time consent is signed through last study visit. If an AE/SAE is ongoing at the end of the reporting period, it should be followed until resolved, stabilized, or a satisfactory explanation is apparent.

9.1.5. Serious Adverse Event (SAE) Reporting

The investigator is responsible for notifying the lead site of any SAE within 24 hours of awareness. The site should report the SAE to their IRB within the required timelines.

9.1.6. Significant but Non-serious Adverse Events:

A case report form will be provided to capture adverse events. The study monitor will review significant but Non-serious adverse events at remote or on site monitoring visits.

9.1.7. Post-Study Adverse Events:

Participants with unresolved previously reported adverse events or new adverse events should be followed by the investigator until the events are resolved, stabilized, the participant is lost to follow-up or an explanation is evident. Resolution means that the participant has returned to baseline state of health or that the investigator does not expect any further improvement or worsening of the adverse event.

9.2 Risks of participation

Although the current study targets healthy subjects who have tinnitus without compromised immune function and risks of participation are expectedly reduced, risks that have been listed in the package insert for Etanercept published by Amgen still apply to the current study. (https://pi.amgen.com/~/media/amgen/repositorysites/pi-amgen-com/enbrel/enbrel_pi.ashx)

9.3 Monitoring to Assess Safety, Contraindications and Warnings/Precautions

Consistent with the guidelines in the package insert prior to initiating Etanercept and at the end of the study patients will be evaluated for active tuberculosis and tested for latent infection.

Enbrel should not be administered to patients with sepsis. According to the package insert, increased risks exist following administration Etanercept

9.4 Drug interactions and cautionary measures:

Subjects receiving Etancercept treatment should not receive live vaccines. Subjects with a significant exposure to **varicella virus** should temporarily discontinue Etanercept therapy and be considered for prophylactic treatment with varicella zoster immune globulin.

In clinical studies, concurrent administration of **abatacept** and Enbrel resulted in increased incidences of serious adverse events, including infections, and did not demonstrate increased clinical benefit. Subjects taking abatacept are excluded from participation.

The use of Etanercept in subjects receiving concurrent **cyclophosphamide** therapy is not recommended. Subjects taking cyclophosphamide are excluded from participation

Subjects in a clinical study who were on established therapy with **sulfasalazine**, to which Etanercept was added, were noted to develop a mild decrease in mean neutrophil counts in comparison to groups treated with either Etanercept or sulfasalazine alone. The clinical significance of this observation is unknown. Subjects on sulfasalazine are excluded from participation.

In the event of an anaphylactic reaction, the study subject will be discontinued from the study and send to the nearest emergency facility.

9.4.1 Expected Adverse Drug Reaction

All adverse drug reactions that may be anticipated are included in the package insert.

9.4.2 Unexpected Adverse Drug Reaction

An unexpected adverse drug reaction is an adverse event where the nature or severity of the reaction or effect is not consistent with the applicable product information provided in the protocol or package insert. In addition, events that add significant information on specificity or severity of known, already documented adverse reactions should be considered “unexpected.”

9.5 Reporting of Adverse Events and Serious Adverse Events

All SAEs must be reported in a timely manner, therefore, it is important that the PI at any site report to the IRB and HRPO, any reportable AEs that by the definition in Section 9.1

would be considered serious, even if the investigator does not consider the AE to be clinically significant or drug-related. All SAEs must be reported to the coordinating center within 24 hours of Investigator awareness and to local IRB and HRPO within their required reporting guidelines. A SAE cover sheet and SAE reporting form MEDWATCH 3500 are to be completed and sent to the lead site. Direct telephone communication is also required to ensure correct procedure. The Investigator must report all SAEs to their IRB per required reporting period. The grant PI will notify all sites of SAEs using the MEDWATCH 3500 form and through DSMB reports.

Investigators will note ADRs on the Adverse Event CRF and must follow subjects with ADRs until the event has resolved, or the condition has stabilized. The PI/Site PIs will report all reportable ADRs to the relevant regulatory agencies as required

9.6 Adverse Experiences with Blood Test

Blood draw is routinely conducted in medical practice. Thus, there will be minimum risk during collection of blood samples.

10.0 EXPLORATORY EFFICACY PARAMETERS

Participating subjects will complete audiological evaluations, VNS, and tinnitus questionnaires. The physician will interview the subject and conduct auditory tests to confirm the questionnaire responses and help determine the severity of tinnitus before, during and after Etanercept administration.

After drug is injected or saline placebo is injected, it is expected that the subject may sense one or more of the following: changes in tinnitus volume, oscillations in tinnitus frequency (pitch) or new frequencies (pitches), very brief intervals of silence, and/or changes in the tinnitus annoyance effect. Due to the delay in time for the drug effects, the effect on tinnitus at different frequencies may differ with the higher frequencies being suppressed first.

During the course of drug administrations, any changes in tinnitus experienced by the subject will be recorded. For all subjects, tinnitus questionnaires and VNS will be completed pre-, during, and post-drug administration.

11.0 STATISTICAL CONSIDERATIONS

Sample size selection

Statistically, power analyses examined the sample size requirements to detect clinically meaningful group differences on each primary outcome (three hypotheses each in Aim 1 and Aim 2). Note, to streamline power analysis and to attenuate the effect of attrition we have chosen four observations which is the minimum of two specific aims (Aim 1 has five while Aim 2 has four observations per subject). Three co-primaries have been proposed in each aim. The smaller of the alpha-levels for the three primaries with the Hochberg adjustment will be 0.0167 (=0.05/3). Therefore, 0.0167 used to estimate the multiplicity

adjusted sample size (Leon 2004). Based on the results, the protocol proposes recruitment of 300 participants (150 per group) to preserve 80% or more power. After accounting for about 5% for attrition, the proposed final sample size is 155/group (total N=310).

Analysis
All AEs will be reported specifying whether they occur during or following drug administration and indicating both severity and physician attribution as to likelihood of association to study drug. Efficacy data will be summarized using descriptive statistics. Data will be explored to determine if the outcomes are consistent with those predicted by the simulations with regard to timing and extent of tinnitus relief depending on the nature of the tinnitus.

MRI Sub-Study
Sample size and statistics.
Two-sided t-test comparing Etanercept treated (Group 1) and Placebo treated (Group 2) will be performed on the MRI variables assessed at baseline. The null hypothesis of no difference between Groups 1 and 2 will be tested against the alternative hypothesis that the Group 2 mean is worse than Group 1 at significance level 0.05.

Analysis will be performed on the change from baseline to 12th and 24th week assessment for each MRI variable. A one-sided paired t-test (or appropriate non-parametric test) will be performed on the change from baseline. The null hypothesis of no change from baseline will be tested against the alternative hypothesis that the mean change is negative (worsening) at significance level 0.05.

For between group comparison of RS-fMRI, SWI and DTI between Etanercept treated and Placebo treated subjects, the difference in means and the standard deviation in default mode network (DMN) and dorsal attention network (DAN) were taken to be $\delta = 0.12$ and $\sigma = 0.2$ for DMN and $\delta = 0.18$ and $\sigma = 0.3$ for DAN.

Based on the estimates, a subset of 40 subjects (20 Etanercept treated and 20 Placebo treated), for a total of three time points will give us a power greater than 80% for a two-sided test at a significance level of 0.05%.

Sub-Study TNF-α concentration
This secondary hypothesis is not powered; rather, the data will be collected for secondary feasibility analysis only.

Because this research is an "intent-to-treat" study, it is important to point out the distinction between withdrawal from treatment and withdrawal from the study. With intent-to-treat designs, subjects who are withdrawn from treatment continue to be followed and have data collected at all scheduled outcome evaluations. This method reduces introducing bias into the estimate of treatment efficacy.

12.0 REGULATORY, ADMINISTRATIVE AND ETHICAL OBLIGATIONS

12.1 Institutional Review Board (IRB)

This trial will be conducted according to the Declaration of Helsinki. The PI/Site PIs will submit the protocol, informed consent, package insert, and any other relevant supporting information to the appropriate IRB for review and approval prior to study initiation. A letter confirming IRB approval of the protocol and informed consent**,** a statement that the IRB is organized and operates according to good clinical practice (GCP) and the applicable laws and regulations, and financial disclosures **must** be forwarded to lead site data coordinating center at Wayne State University. Participating sites must use the versions or the protocol and other study documents provided by the lead site; study documents other than the informed consent form may not be modified. The participating site also must forward a signed Investigator Agreement / Protocol Signature Page to Wayne State University prior to screening and enrolling subjects for study enrollment.

The Principal Investigator will be responsible for informing the regulatory authority and the IRB of any amendments to the protocol. Prior to implementation of changes to the study, protocol amendments must also be approved by the IRB and local regulatory agencies, the signed Investigator Agreement / Protocol Signature Page along with IRB approval must be provided to Wayne State University.

Upon completion of the study, a summary of the data related to investigational products will be prepared and submitted to the regulatory authority.

12.2 Human Research Protection Office (HRPO)

HRPO at US Army Medical Research and Material Command (USAMRMC) is responsible for conducting the following activities:

- Principal advisor to the Command for human subjects protection.
- Develop and implement human subjects protection policies & regulations.
- Maintain the USAMRMC Volunteer Registry Management System.
- Review and approve intramural and extramural human subjects protocols.
- Conduct human subjects protection site visits.

12.3 Study Compliance

This study will be conducted in compliance with the protocol, principles of International Conference on Harmonization (ICH), good clinical practice (GCP), Declaration of Helsinki, and all applicable national regulations governing clinical trials.

12.4 Informed Consent

A copy of the IRB approved informed consents will be forwarded to HRPO for regulatory purposes. The investigator or designee **must** explain to each subject the purpose and nature

of the study, study procedures, possible adverse effects, and all other elements of consent as defined in 21CFR §50 (for US sites), and applicable national and local regulations governing informed consent. Each subject must provide a signed and dated informed consent prior to enrollment into this study. The PI or designee must sign and date the informed consent form after the subject, but before any study procedures are performed. Original signed consent forms must remain in each subject's medical records and be available for verification by study monitors at any time. A copy of the informed consent will be given to the subject for his or her own records.

In accordance with local and national privacy regulations, the Investigator or designee **must** explain to each subject, prior to screening, that for the evaluation of study results the subject's protected health information obtained during the study may be shared with regulatory agencies such as FDA, IRBs, and HRPO. Wayne State University and its collaborating institutions will not use the subject's protected health information or disclose it to a third party without applicable subject authorization. It is the responsibility of the Investigator or the Investigator's designee to obtain, from each subject, written permission for use of protected health information. If a subject withdraws permission to use protected health information, it is the Investigator's responsibility to obtain a written request from the subject **and** to ensure that no further data will be collected from the subject. Any data collected on the subject prior to withdrawal will be used in the analysis of study results.

The informed consent has been developed by Wayne State University and its collaborating institutions. A copy of the form will be provided to the PI/Site PIs for submission and approval by the Institutional Review Board (IRB). A copy of the informed consent **must be submitted** to the **Wayne State University** lead site **for approval prior to site IRB submission**. All subjects must sign the informed consent before any study procedures, including screening, are performed.

12.5 Regulatory Agencies

Wayne State University and its collaborating institutions will conduct this clinical trial in accordance and agreement with the rules and regulations of the local regulatory authority. A local representative will be appointed to monitor the clinical trial and to communicate with the regulatory agency.

12.6 Conduct of Study and Protection of Human Subjects

The Principal Investigator and site Principal Investigators must ensure that:

- He or she will personally conduct or supervise the study.
- His or her staff and all persons who assist in the conduct of the study clearly understand their responsibilities and have their names included in the Study Staff Signature and Delegation of Authority log. The Investigator will sign the authorization log whenever it is updated with new responsibilities or staff membership.
- The study is conducted according to the protocol and all applicable regulations.

- The protection of each subject's rights and welfare is maintained.
- Signed and dated informed consent and HIPAA authorization/permission to use protected health information are obtained from each subject prior to conducting study procedures. If a subject or subject's legal guardian withdraws permission to use protected health information, the investigator will obtain a written request from the subject or subject's legal guardian, and will ensure that no further data be collected from the subject.
- The consent process is conducted in compliance with all applicable regulations and privacy acts.
- The IRB and HRPO complies with applicable regulations and conducts initial and ongoing reviews and approvals of the study.
- Any amendment to the protocol is submitted promptly to the IRB and HRPO.

13.0 RECORD KEEPING & MONITORING

13.1 Record Retention

The investigator must retain a copy of all documents relating to this clinical trial for a minimum of 5 years after the study is closed. The investigator must retain the documents for a longer period when required by other applicable requirements. Essential documents shall be archived in a way that ensures that they are readily available, upon request, by the competent authorities or appropriate regulatory authorities. The medical files of clinical trial subjects shall be retained in accordance with national legislation and the maximum period of time permitted by the hospital, institution, or private practice. The investigator is responsible for contacting the lead site before the destruction of any study-related documents and must wait for written approval from lead PI before destruction may proceed.

The medical and clinical monitoring representatives will conduct inspections of study-related data, CRFs, subject medical records, and source documentation in accordance with GCP. Regulatory authorities may conduct similar inspections.

13.2 Case Report Forms (CRFs) and Source Documents

The Wayne State University Coordinating Center (PI's institution) will supply each site with source workbooks. Data from source will be entered into the Wayne State University Electronic Data capture system. Each site will be trained on the EDC system and provided instructions for their completion. Original source documents and workbooks will be located at the investigational site. Participating sites will be asked to upload de-identified source for remote monitoring.

Study site personnel will accurately and legibly complete individual source workbooks in indelible **black ink** and are to be reviewed and signed by the investigator. All corrections to entered data will be made by drawing a single line through the information to be

corrected, without obscuring it. All corrections will be initialed and dated (and explained, if necessary). **Do not use "white-out" or obscuring correction fluid/tape**. The investigator will ensure that the source are accurate, complete, legible, and timely.

CRF entries will be entered directly into the database. The principal investigator will be responsible for reviewing and digitally signing off on the data for accuracy and completeness.

Subjects that have signed the Informed Consent are considered enrolled. Subjects that did not receive study medication for reasons other than an adverse event will be considered as a screen fail. A Screening CRF and Study Termination CRF will be completed for screen fail subjects.

13.3 Monitoring

The purpose of monitoring is to ensure study participant safety, compliance with the protocol, and the quality and integrity of the data. Monitoring will be conducted through a combination of on-site and remote visits with the investigator and site staff, as well as any appropriate communications by mail, fax, e-mail, or telephone. Monitoring for this study will focus on safety and outcome measure data. Initial data review will occur upon completion of the Baseline visit via remote access. This study is subject to Quality Assurance reviews and/or audits by an Independent Monitor, as required per DoD policy.

Every effort will be made to maintain the anonymity and confidentiality of all subjects during this clinical study. However, because of the experimental nature of this treatment, the Investigator and PI/Site PIs agree to allow the lead site Wayne State University, IRB/HRPO, and authorized employees of the appropriate regulatory agencies to inspect the facilities used in this study and, for purposes of verification, allow direct or over the shoulder access to the hospital or clinic records of all subjects enrolled in this study. A statement to this effect will be included in the informed consent and permission form authorizing the use of protected health information.

13.4 Regulatory Inspections

Regulatory authorities, Wayne State University lead site and the site's local regulatory body or IEC may conduct regulatory inspections.

13.5 Site Discontinuation

The DoD as the Sponsor, HRPO, the coordinating center of local IRB, Wayne State University lead PI has the right to terminate a site at any time. Reasons for terminating a site's participation may include, but are not limited to, the following:

- Subject enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.
- Investigators at any sites that do not adhere to the protocol or applicable regulatory guidelines in conducting the study.

13.6 Executive Committee

An executive committee composed of experts in tinnitus trials, blast-induced tinnitus, trial conduct, military-related tinnitus trials, statistician and TNF-α therapy developer will hold quarterly teleconferences while communicating routinely with all Site PIs to coordinate sites' trial activity oversight. The committee review recruitment, drug administration, subjects' safety and benefits, outcome measures, FDA/IRB/Human Research Protection Office (HRPO) compliance, data management, and prepare publications. The executive committee will also provide interim analysis about ongoing drug treatment and outcome measurements.

13.7 Data Safety Monitoring Board

The study will be monitored by an independent Data Safety Monitoring Board (DMSB). The DSMB members will consist of five independent experts on the study medication, tinnitus, audiology, conducting clinical trials, and a statistician, none of whom will be otherwise involved in the conduct of study.

Safety analyses will be conducted after the first 25 patients have been enrolled or 6 months after the study opens for enrollment, whichever comes first, and will be done every 6 months for the duration of the study. This schedule may be adjusted if any safety issues are identified. The DSMB will monitor the incidence of the anticipated adverse events, as well as the overall safety of patients, during the study. Safety will be assessed through descriptive summaries of adverse events, vital signs, safety labs, and other protocol specified tests being done for safety. The study biostatistician will do analyses as per data analysis plan and blinded results will be forwarded to the DSMB for review in open and closed session reports. The Data Safety Monitoring Board may, at any time, recommend terminating the study because of safety concerns or advise the study PI at any site if the data suggest that the study should be modified. The Data Safety Monitoring Board may also recommend that additional safety monitoring be done or that additional meetings be held. There are no formal boundaries for early stopping based on safety or efficacy. The DSMB will consider clinical narratives and statistical results when considering safety related issues. There are no provisions for stopping based on efficacy and it is expected the trial will enroll until completion.

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